Development of immunomodulatory biomaterial in tissue repair

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Date: 04/07/15

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INTRODUCTION

More than 8.9 million bone fractures annually worldwide resulting in the occurrence of a fracture every three seconds (1). many treatments can be performed to manage different types of fracture, such as non-surgical stabilization by using wood stick wrapped around fracture limb or surgical bone graft for bone loss or severe trauma. Regardless of the treatment strategy, the ultimate goal after injury is to return the damaged tissue back to its original state in order to restore functionality. Unfortunately, according to abbreviated injury scale (AIS), the maximal level of fracture is currently untreatable. Today, the development of biomaterial is investigating a new way to handle untreatable damage. For example, polymer application and surface coating technology have influenced most methods in drug delivery. In this research, PLGA bone coating will be used to investigate the functionality of tissue proliferation. Acute inflammation is the on of the first stages of the body's response to injury. Signals produced by damaged tissue relays information almost immediately after an injury, causing a series of wound-healing responses including vasodilation (temporarily increasing vessel diameter, redness) and influx of protein-rich fluid and cells (swelling) (3) . Cytokines, or small signaling proteins, released from activated mononuclear leukocytes play an important role that affects the behavior of neighboring cells. (2) In this research, Inflammatory (M1) and anti-inflammatory (M2) macrophages were studied to understand the mechanism of cellular bone formation and defense at the fracture site

Bone healing is very complicated process involving proliferation of tissue cells and stem cell differentiation. A complex cascade of chemical signals and cells, such as growth factors, inflammatory cytokines, osteoclasts (cells that break down bone), and osteoblast (bone-forming cell), work as a team to perform a compact task. It seems like our body has a mysterious program that leads protein and cell present to accomplish different tasks. The injury site initiates inflammation and spreads a signal to recruit inflammatory monocytes. The inflammatory monocytes infiltrate the injury site and work to effectively defend and clear the virus, bacterial, and other infection from an external source. However, the functionality of inflammation can also lead to pathogenesis of inflammation and degenerative disease (2). Therefore, anti-inflammatory monocytes patrolling in the vasculature move from the blood vessel to the tissue and differentiate into anti-inflammatory macrophages called “M2” macrophages. Anti-inflammatory macrophages clean the inflammation and also repair damaged tissues (3). Ideally, if we can identify the correlation within those components and speed up the procedure, we could increase the body’s regeneration rate. The previous study has shown the efficiency of FTY720, The US Food and Drug Administration (FDA) approved in 2010, promoting local vascular formation and grafted bone bridging. (4) Moreover, the sphingosine 1-phosphate (S1P) receptor agonist FTY720 therapeutically affect on lymphocytes and vascular endothelium. (5) Therefore, our research focuses on the combination of coating FTY720 in

PLGA and developing strategies to increase the efficiency of recruitment of therapeutic cell types.

EXPERIMENTAL DESIGN/METHODS

We performed a rat tibial defect to study the contribution of inflammatory cells to fracture repair. Three different treatments were investigated: uncoated allografts, PLGA-coated allografts, and PLGA-coated FTY720 drug, were implanted in defect created in the rat tibial bone. PLGA is an FDA-approved copolymer that is commonly used for in vivo drug delivery because of the ability to control the rate of degradation and drug . PLGA can store the drugs and slowly degrade to release them to the targeted tissue. FTY720 has been demonstrated to have the ability to promote vascular network expansion that supports tissue regeneration (6). Good tissue regeneration provides a better environment to stably grow and remodel bones (7)(8). The hypothesis is that the drug treated implanted bone can successfully deliver and stably release FTY720 to promote better bone formation in the deflect site. 8 weeks after the implantation surgery, the tibias from three groups were scanned by microCT to see the inside structure and the progress of the bone formation. MicroCT scanning provides detail cross-section images of rat tibial bones. The image showed the condition of bone fracture healing between the object's tibial bone and implanted bone. Rats were divided into three groups, uncoated, PLAGA, and FTY. At week 8, the implanted bone was determine the condition of bridging to the object's bone at each of four locations on implanted bone: top/left, top/right, bottom/left, and bottom/right. Moreover, the longest and

shortest gap length from each implanted bone to the object's bone was measured. Both of the longest and shortest values are zero if the bone forms a bridge (bone connected). One-way ANOVA was performed to analyze the longest and shortest length difference within three groups. The tibias were sectioned and placed on the slides to study its histology. CD206 and CCR7 antibodies were used to study the inflammatory response. We would like to know the amount of inflammatory macrophages (M1) and anti-inflammatory macrophages (M2) in this stage. Moreover, we would like to know whether those M1 and M2 were resident cells or came from bone marrow. Therefore, GFP bone marrow from the chimera rat was transplanted to our experimental rat, so the new form blood cells from FTP bone marrow expresses GFP after histology staining. The slides were also stained with antibodies, osteocalcin, and α-SMA to observe the distribution of bone-forming cells and blood vessel cells, respectively.

Results

MicroCT scanning showed the average longest gap in uncoated group was 0.290mm, in PLAGA group is 0.074mm, and FTY720 group 0.091mm. ANOVA test showed that the gap length has significant difference in uncoated group with p-value 0.0002 (fig.1). The average shortest gap in uncoated group is 0.097mm, in PLAGA group was 0.040mm, and FTY720 group was 0.034mm. ANOVA test showed that the gap length has significant difference in uncoated group with p-value < 0.0001 (fig.2). Uncoated group had 2 sites

showing bone bridge formation, PLAG group had 6, and FTY720 group had 11. Figure 1 and figure 2 have shown that the implanted bone in FTY720 treated group actively grows toward host bone. M1 and M2 Macrophage that generated from chimera rat's bone marrow were labeled by CD206+/GFP+ and CCR7+/GFP+, respectively. The statistic analysis show that a little more CCR7+/GFP+ labeled cells presented in PLGA-only group and almost the same amount of CCR7+/GFP+ labeled cells presented in control and FTY720 group. The amount of CCR7+/GFP+ labeled cell has no significant difference within three groups. (fig.3) More CD206+/GFP+ labeled cells presented PLGA and FTY720 group. (fig.4) However, the amount of CD206+/GFP labeled cells didn't show the significant difference. To estimate how FTY720 promote bone-derived cells differentiate to M1 and M2 macrophage, the tibial bone tissue was observed under confocal microscope at week 2 to the distribution of CD206+/GFP+ and CCR7+/GFP+ cells. (Fig.5,6). The analysis show that CCR7+ cells has higher percentage that also labeled GFP+ than CD206+ cells. Therefore, more CCR7+/GFP+ cells derived from bone marrow. (Fig.7)

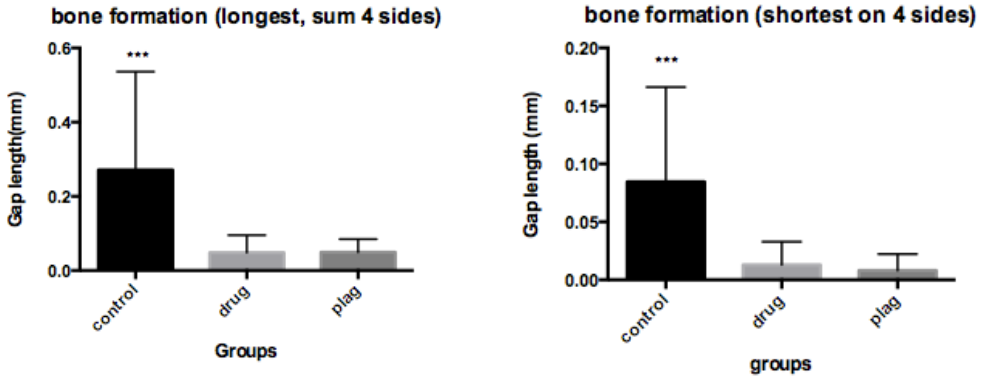
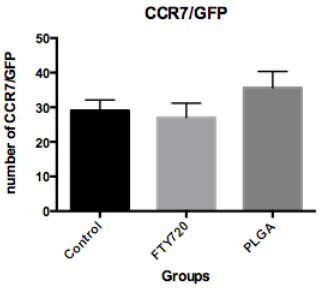


Fig.1 ANOVA test Gap length within uncoated, PLGA, and FTY. ANOVA test showed the gap length has significant difference in uncoated group with p-value 0.0002

Fig.2 ANOVA test Gap length within uncoated, PLGA, and FTY. ANOVA test showed that the gap length has significant difference in uncoated group with p-value < 0.0001



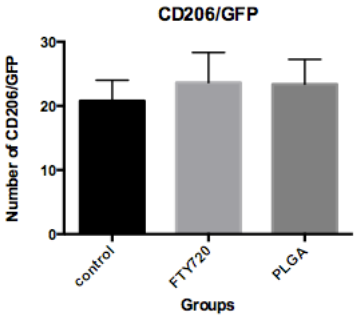


Fig.3 number of cells labeled with CCR7 and GFP, which shows M2 macrophage generated from bone marrow. The amount of CCR7+/GFP+ labeled cell has no significant difference within three groups.

Fig.4 number of cells labeled with CD206 and GFP, which shows M1 macrophage generated from bone marrow. the amount of CD206+/GFP+ labeled cells didn't show the significant difference

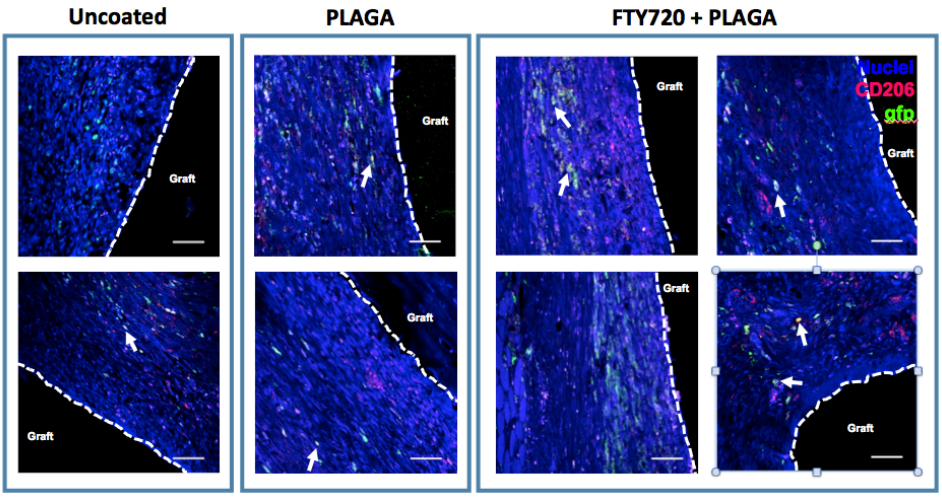


Fig 5. Induce recruitment of bone marrow-derived macrophages (M1) by FTY720. At week 2, rat tibial bone sections were stained with anti-GFP, DAPI, and CD206. The images were taken under confocal microscope with Scale bars = 50 μm. The dot white line represents the edge of graft bone.

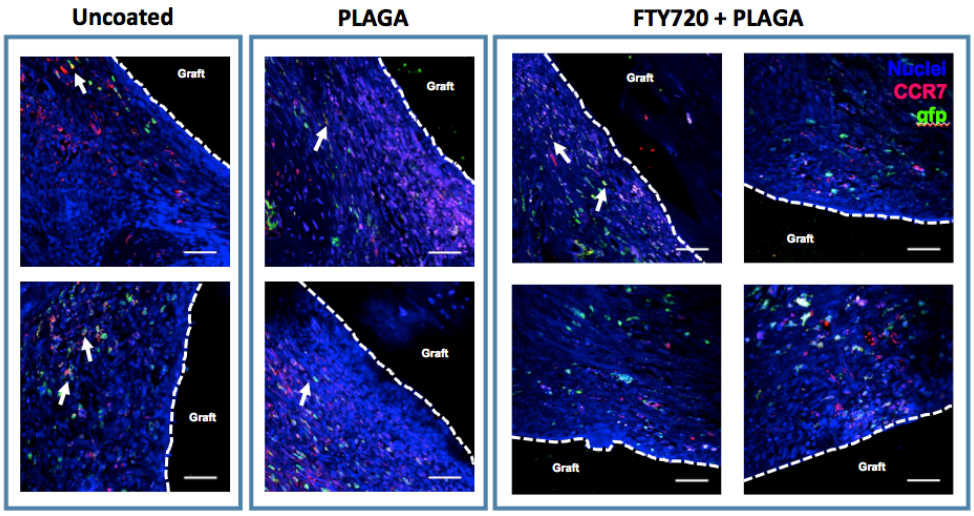


Fig 6. Induce recruitment of bone marrow-derived macrophages (M2) by FTY720. At week 2, rat tibial bone sections were stained with anti-GFP, DAPI, and CCR7. The images were taken under confocal microscope with Scale bars = 50 μm. The dot white line represents the edge of graft bone.

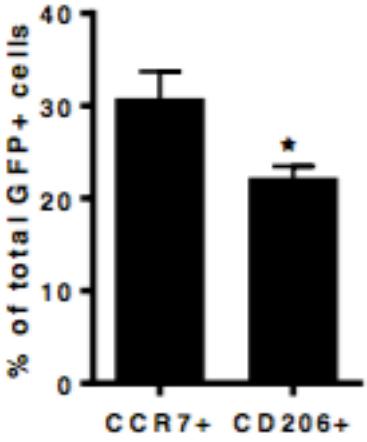


Fig.7. The analysis of the percentage of CCR7+/GFP+ and CD20+/GFP+ cells in total GFP+ cell population.

DISCUSSION AND FUTURE WORK

The microCT showed the result that both of coated and FTY720 treated group form significantly more bridges from the implanted bone to the host bone. Furthermore, we measured the shortest and longest gap between both bones to see if FTY720 reduces the distance between host and donor bone. The average gap distance of FTY720 group was less than the PLGA coated group but it was not significant due to insufficient sample group size. The bone section slides were observed using confocal microscopy. The CD206 (M1) and CCR7 (M2) cells were qualified in each of the slides. The ratio of M1 and M2 containing GFP were found. However, the final analyses don't have significance due to small sample size. So far, these results have demonstrated that FTY720 can increase bone formation through altering cell recruitment into the healing bone. The development of bone coating with FTY720 could provide better outcome in bone grafting surgery and investigate FTY720 functionality that better promoting defect bone healing. The results showed that FTY720 coated could stimulate bone formation, and we had successfully recruited more monocyte to the targeted site, but the fate of inflammatory and anti-inflammatory monocytes is still unknown. In conclusion, while our results are showing positive significance, further research will be continue on studying where monocytes came from and where they go after the bone formation.

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